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Mean geocarposphere temperatures that induce preharvest aflatoxin contamination of peanuts under drought stress*

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Abstract

Apparently undamaged peanuts grown under environmental stress in the form of drought and heat become contaminated with *Aspergillus flavus* and aflatoxin in the soil prior to harvest. The upper mean temperature limit for aflatoxin contamination in undamaged peanut kernels grown under drought stress the latter 4–6 weeks of the growing season was between 29.6–31.3 ° C. The lower limit was between 25.7–26.3 ° C. That is, peanuts grown under drought stress with a mean geocarposphere temperature of 29.6 ° C were highly contaminated while those at 31.3 ° C were not contaminated. Likewise, those grown under drought stress with a mean geocarposphere temperature of 25.7 ° C were not contaminated while those subjected to a mean geocarposphere temperature of 26.0 ° C resulted in some categories becoming contaminated. Increasing the mean temperature up to 29.6 ° C caused increasing amounts of contamination.

Introduction

Previous studies have shown an association between drought stress in peanuts and increased aflatoxin contamination (6, 7, 10–13). Studies using novel experimental plots designed to monitor soil moisture and temperature have shown that the major environmental factor involved in preharvest invasion of peanuts with *Aspergillus flavus* and *A. parasiticus* and subsequent contamination with aflatoxin is extreme and prolonged drought stress (1–3, 8, 12). A possible role of drought stress in preharvest aflatoxin contamination is to eliminate microbial competitors of *A. flavus,* while elevating the soil temperature in the peanut geocarposphere (approximately 3–5 cm below soil surface). This latter phenomenon occurs when the peanut canopy recedes during severe and prolonged drought stress allowing solar radiation to reach to the soil surface thus elevating the soil temperature in the geocarposphere.

It was established that a mean threshold geocarposphere temperature (drought stress the last 4–6 weeks of the growing season) of 25.7–27.0 ° C was required for aflatoxin contamination (2). Peanuts grown under drought stress and subjected to mean geocarposphere temperatures below 25.7 ° C (in the absence of insect or other damage) were not contaminated with aflatoxin. However, those grown at geocarposphere temperatures at or above 27 ° C were likely to become contaminated in the absence of visible damage (2, 3).

The purpose of this study was to define more accurately the upper range of mean geocarposphere temperature requirements for preharvest aflatoxin contamination of drought-stressed peanuts.

Materials and methods

Environmental control plots

This study was conducted with six environmental

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control plots designed to accurately monitor and control soil temperature and moisture in the peanut geocarposphere (1, 2) (data were collected every two hours throughout growing season). The selected treatments, which were initiated 91 days after planting (DAP), included an irrigated control (plot 1 provided optimum moisture throughout the growing season) and five drought treatments (plots 2–6) with temperature modification designed to provide mean geocarposphere temperatures of 24 ° C (plot 2), 26 ° C (plot 3), 28 ° C (plot 4), 30 ° C (plot 5) and 32 ° C (plot 6) throughout the treatment period of 91–138 DAP.

Each plot was completely encased in a drainage bed of gravel to prevent lateral movement of soil moisture into the facility. Moisture from precipitation was excluded from the plots by moisture sensor-equipped mechanized roofs that automatically closed to cover the plots at the inception of precipitation (1, 2). Irrigation was provided to the plots when moisture tension reached 0.2 bar as measured with a tensiometer (Irrometer Company, Riverside, CA) at a soil depth of 30 cm. All plots were provided with adequate soil moisture for 91 days after planting, when the different regimens were imposed. Soil moisture tension under and between the rows at 5, 30, and 60 cm below the surface was measured with Delmhorst gypsum blocks (Delmhorst Instrument Co., Boonton, N.J.) throughout the growing season. In each plot, there were at least 10 moisture sensors at each depth. Geocarposphere temperatures in two of the drought treatment plots were elevated to desired temperatures using thermostatically-controlled, lead-shielded heating cables arranged 10 cm apart and placed at a depth of approximately 12.7 cm. Three drought treatment plots were equipped with 6.35-mm copper tubing coated with chemically-resistant epoxy paint, and water was circulated through the coils periodically to reduce soil temperature in the geocarposphere. Moisture and temperature data were collected automatically every two hours on cassette tapes using a 500 channel data collection system (Monitor Lab Model 9302, San Diego, Calif.). Data were analyzed using Statistical Analysis System (SAS 79).

Cultivation of peanuts

The research plots contained 'Tifton loamy sand' soil; each plot was analyzed for major and minor plant nutrients by Waters Agricultural Laboratory and Consulting Company, Camilla, GA 31730. Adjustments in fertility were made as needed. The peanut variety used in this study was the Florunner cultivar, since this is the most widely grown variety, especially in the southeastern peanut belt. Florunner peanuts were planted on April 28, 1982, using a 92 cm row pattern. Fungicides, herbicides, and insecticides were applied, as necessary, at the manufacturer's recommended rates. Spray applications of Bravo (chlorothalonil) to control Cercospora leafspot were made on May 26, June 9 and 22, July 2, 15 and 28, and August 6 and 23. On April 23, the pre-plant herbicide Dual (metolachlor) was applied, followed by a pre-emergence application of Lasso (alachlor) and Dyanap (naptalam and dinoseb) on May 20. Insecticide applications were: Temik (aldicarb), was placed in the furrow at planting (April 28); Dyfonate granular (fonofas) on July 20 to control lesser cornstalk borer (*Elasmopalpus lignosellus*); Sevin on July 15 to control corn earworm (*Heliothis zea* [Boddie]); and Kelthane on August 9 and 13 to control spidermites (*Tetranychus* spp.).

Harvesting, shelling, and grading of peanuts

Peanuts from all the plots were dug by hand 138 DAP. Those peanuts from plot 1 (irrigated) were removed from the vines and shelled manually because of the relatively high moisture content. Peanuts from the other five drought plots were harvested with a plot-sized combine, and shelled with a Model 4, National Peanut Research Laboratory sample sheller (5) and screened into commercial grade categories prior to determination of degree of mold invasion and aflatoxin contamination.

The grade categories, primarily based on size, were jumbo, medium, number 1, other edible, and oil stock. Those peanuts that were shelled during combining (loose shelled kernels = LSK) for each treatment were separated from unshelled peanuts prior to shelling and analyzed as a distinct category. Damaged kernels were hand-picked and separated from all grade categories, mixed, and also analyzed as an additional category.

Assessment of the microflora

Numbers and kinds of fungi within peanut kernels at harvest (138 DAP) were estimated by plating out surface-sterilized (0.5% sodium hypochlorite solution [Clorox], 5 min) kernels on 2% malt ex-

tract agar with and without 10% NaCl followed by incubation at 25 °C and 37 °C. Fungi were identified to genus and species, with emphasis on *Aspergillus* and *Penicillium* species. Actinomycetes and bacteria were recorded but not classified in most cases. Soil, rhizosphere, and geocarposphere microflora were also studied. Results, presented here, are only for *A. flavus* group fungi.

Aflatoxin analyses

Peanut samples were analyzed for aflatoxin using the minicolumn method of Holaday and Lansden (9) followed immediately by quantitative analyses using high-pressure liquid chromatography (HPLC) (4). The HPLC technique utilized a radial compression module containing a Radial-Pak silica cartridge (Waters Associates, Milford, Mass.) and a solvent system consisting of water-saturated chloroform supplemented with 0.6% reagent alcohol (Fisher Scientific Co., Atlanta, Ga.). The aflatoxins were detected with a Kratos Model FS 950 Flouromat Fluorometer detector.

Results and discussion

The treatment strategy was to induce drought stress 91 DAP in all the treatments, except in the irrigated control, and to vary the overall mean geocarposphere temperature in the drought treatments by 2 °C increments, starting at 24 °C and increasing to 32 °C. Table 1 shows the mean soil moisture tension for each treatment in the geocarposphere region during the treatment period. The mean bars tension in plot 2 was slightly lower than the other drought treatments; however, a mean moisture tension of 9.8 bars represents a severely dry environment biologically, and the difference between this value and the values for the other drought treatments was not considered significant in this study. Table 1 also shows the mean geocarposphere temperatures for the different treatments. The overall mean geocarposphere temperatures achieved in the drought treatments were near the target temperatures desired for the experiment. The mean geocarposphere temperature for the irrigated control did not vary significantly (25.6 °C compared to 25.2 and 23.9 °C, respectively) from the analogous irrigated treatments for experiments conducted the two previous years (CY 1980 and 1981) (2, 8). This is in contrast to corresponding ambient temperatures for the same treatment periods, especially during 1980 vs. 1981 and 1982. The mean ambient temperatures for CY 1980 during

Table 1. Mean geocarposphere temperatures and mean soil moisture data during the treatment period (91–138 DAP) for six treatments.

Plot/Treatment	Bars tension Mean	Geocarposphere temp., °C		
		Mean High	Mean	Mean Low
1/Irrigated	2.1	27.8	25.6	24.5
2/Drought	9.8	34.9	31.3	28.8
3/Drought	14.2	33.2	29.6	26.6
4/Drought	14.9	31.4	27.8	25.6
5/Drought	12.2	29.8	26.3	24.3
6/Drought	13.0	28.0	24.6	22.8

Table 2. Incidence of the *Aspergillus flavus* group on peanut kernels grown under varying soil temperature and moisture.

Mean temp. °C during treatment period	Treatment Irrigated Drought with modified soil temperatures					
	25.6 °C	24.6 °C	26.3 °C	27.8 °C	29.6 °C	31.3 °C
Kernel category	(percent kernels colonized)					
Jumbo	26.4	11.1	45.9	11.8	37.1	38.0
Medium[a]	70.4	17.8	18.1	72.0	84.9	26.5
#1	36.2	23.9	20.0	35.0	88.6	89.3
Other edible	39.0	33.3	55.7	60.0	68.0	73.3
Oil stock[a]	16.8	33.3	60.0	67.7	75.7	98.0
LSK	53.3	30.0	65.0	92.0	80.0	100.0
Damaged	88.0	65.2	86.7	28.6	68.0	68.6

[a] Most representative figures in terms of numbers of kernels available for assessment.

August and September (critical period for preharvest aflatoxin contamination) were 28.3 and 26.8 ° C, respectively, while the mean ambient temperatures for the corresponding months during 1981 and 1982 were 25.8 and 23.9 and 26.1 and 23.8 ° C, respectively. There was also considerably less solar radiation during this period in 1981 as compared to 1980 (2). These data demonstrate that the mean geocarposphere temperatures in peanuts that are provided with adequate moisture during the latter half of the growing season is relatively independent of ambient temperature presumably due to shading of the soil surface by the peanut canopy.

The proportion of kernels colonized by the *Aspergillus flavus* group (*A. flavus* Link and *A. parasiticus* Speare) at harvest varied greatly for the different categories of edible and non-edible oil stock peanuts both within and among treatments (Table 2). However, for kernels from drought-stress treatments, colonization generally was greater with higher soil temperatures during the treatment period except for the warmest treatment (31.3 ° C) where there was less colonization for the 'medium' edible size category. Medium sized kernels comprised about 50% by weight of the total yield of edible kernels and therefore are the most representative category. Colonization by the *A. flavus* group of medium kernels was minimal (17.8% of kernels colonized) for the coolest, drought-stress treatment and maximal (84.9%) with a mean soil temperature of 29.6 ° C. There was a sharp decrease in the proportion of kernels colonized in the warmest treatment (26.5% at 31.3 ° C) (Table 2). The pattern of kernel colonization in non-edible, drought-stress peanuts was the same as that for edible kernels, except that there was no decrease in colonization in the warmest treatment. Also, as expected, a greater proportion of non-edible than edible kernels was colonized by the *A. flavus* group (Table 2).

Tables 3 and 4 compare the aflatoxin content of the various commercial categories of peanuts from each of the six treatments. Again, as in the two previous crop years, the sound kernels from the irrigated treatment were negative for aflatoxin. These data are supported by Wilson *et al.* (14) using similar research plots (no temperature monitor) which showed that drought stress alone did not consistently produce field aflatoxin contamination. In some years other environmental factors must interact with drought stress to promote or inhibit preharvest aflatoxin contamination. However, no significant aflatoxin contamination was detected in any of the treatments in any of the four years where irrigation was applied during the last 40 days of the season. The data in Tables 3 and 4 also indicate that a mean geocarposphere temperature of 31.3 ° C was too high for aflatoxin development in sound kernels. However, mean geocarposphere temperatures of 29.6 ° C, 27.8 ° C and 26.3 ° C were conducive for aflatoxin development, with 29.6 ° C being highly conducive. The mean geocarposphere temperature of 24.6 ° C in the drought treatment of plot 6 was apparently at or near the lower limit for aflatoxin development, since only the other edible category contained low levels of aflatoxin contamination.

Table 3. Aflatoxin content of various commercial grade categories for six treatments.

	Soil treatment/temperature[a]					
Kernel category	I[b] 25.6 ° C	D[b] 24.6 ° C	D 26.3 ° C	D 27.8 ° C	D 29.6 ° C	D 31.3 ° C
Aflatoxin conc. (ppb)						
Jumbo	0	0	0	0	275	0
Medium	0	0	0	2	10	0
#1	0	0	83	250	3 100	0
Other edible	0	20	330	0	660	0
Oil stock	0	0	360	220	440	0
LSK	0	0	0	0	40	0
Damaged	0	(-)[c]	(-)	(-)	6 500	0

[a] Mean geocarposphere (5 cm) temperature (91–138 DAP).
[b] I = Irrigated treatment; D = drought treatment.
[c] (-) No damaged peanuts available for analysis.

Table 4. Aspergillus flavus group colonization and aflatoxin content of peanuts from six treatments.

Plot treatment	Mean temperature °C	Kernels colonized		Aflatoxin	
		Edible %	Non-edible %	Edible ppb	Non-edible ppb
I	25.6	43.0	52.7	0	0
D	31.3	56.8	88.9	0	0
D	29.6	69.7	74.6	380	312
D	27.8	44.7	62.8	29	143
D	26.3	34.9	70.6	45	234
D	24.6	21.5	42.7	0	0

These data show that a mean geocarposphere temperature between 24.6 ° C and 26.3 ° C represents the lower temperature threshold and a mean geocarposphere temperature between 29.6 ° C and 31.3 ° C represents the upper temperature threshold for aflatoxin development in peanuts under drought stress (Table 3).

In irrigated peanuts, a surprisingly large proportion of edible grade kernels was colonized by the *A. flavus* group (mean, 43%) (Table 4). There was, however, no aflatoxin production in edible or non-edible irrigated peanuts. Aflatoxin tests also were especially negative for all categories of peanuts from both the coolest and warmest drought-stress treatments. The greatest concentration of aflatoxin was found in edible peanut kernels with the most *A. flavus* group colonization. Nevertheless, a considerable amount of colonization of peanut kernels by the *A. flavus* group could occur, without aflatoxin production, under irrigation or when the soil temperature was decreased below 25 ° C or increased above 30 ° C (in drought-stressed peanuts). These results indicate that invasion of peanuts by the *A. flavus* group occurred as a separate event from aflatoxin production. This method of mycoflora evaluation did not measure the degree of growth after invasion; therefore, invasion by *A. flavus* appeared to be innocuous, except when environmental stresses in the form of drought and heat were imposed. This suggests that some inherent mechanism imparting resistance to aflatoxin development (in response to increased growth of the fungus after invasion) broke down under temperature stress.

The following conclusions can be drawn from this and previous data (2, 8) obtained in these environmental control facilities: (1) apparently 24 undamaged peanuts grown under environmental stress in the form of drought and heat become contaminated with *A. flavus* and aflatoxin in the soil prior to harvest, (2) sound kernels from peanut plants grown under adequate moisture are not likely to become contaminated with aflatoxin, (3) the mean geocarposphere temperature during the latter 4–6 weeks of the growing season for peanuts grown with adequate moisture is relatively independent of ambient temperature, and (4) the upper temperature limit for aflatoxin formation in undamaged peanut kernels grown under drought stress is between 29.6 and 31.3 ° C, while the lower limit is between 25.7 and 26.3 ° C, therefore, peanuts grown under drought stress may not be contaminated with aflatoxin unless drought is accompanied by elevated geocarposphere temperatures during the latter part of the growing cycle.

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